

MRID No. 413158-02

## DATA EVALUATION RECORD

1. **CHEMICAL:** Imazapyr. Shaughnessey Number: 128829. ? 128821 = AC 243,997  
= ACID
2. **TEST MATERIAL:** AC 243,997; Lot No. AC 4866-62; 99.5% active ingredient; a white powder.
3. **STUDY TYPE:** Mollusc 96-Hour, Flow-Through Shell Deposition Study. Species Tested: Eastern Oyster (*Crassostrea virginica*).
4. **CITATION:** Ward, G.S. 1988. Acute Toxicity on New Shell Growth of the Eastern Oyster (*Crassostrea virginica*). ESE Report No. 87384-0400-2130. Performed by Environmental Science & Engineering, Inc., Gainesville, FL. Submitted by American Cyanamid Company, Princeton, NJ. EPA MRID No. 413158-02.
5. **REVIEWED BY:**  
 Rosemary Graham Mora, M.S.  
 Associate Scientist  
 KBN Engineering and  
 Applied Sciences, Inc.  
 Signature: *Louis M. Rifici for RGM*  
 Date: *6/19/92*
6. **APPROVED BY:**  
 Louis M. Rifici, M.S.  
 Associate Scientist  
 KBN Engineering and  
 Applied Sciences, Inc.  
 Signature: *Louis M. Rifici*  
 Date: *6/19/92*  
 Henry T. Craven, M.S.  
 Supervisor, EEB/EFED  
 USEPA  
 Signature: *Michael D. Craven*  
 Date: *6/26/92*  
*Daniel D. Craven*  
*6-30-92*  
*Henry T. Craven*  
*6/30/92*
7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a 96-hour flow-through mollusc shell deposition study. The control oyster growth (1.36 mm new shell deposition) did not meet the guideline requirements. Flow rate to the test organisms was 21% of the recommended rate and no supplemental food was provided. Based on mean measured concentrations, the EC<sub>50</sub> for eastern oysters exposed to AC 243,997 was >173 mg a.i./l, therefore, AC 243,997 is classified as practically non-toxic to *Crassostrea virginica*. The NOEC was 109 mg a.i./l.
8. **RECOMMENDATIONS:**



2046198

Note, approved to  
 Supplemental as per  
 EEB review under variance  
 D199343. N/Cut  
 08-23-95

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Eastern oysters (*Crassostrea virginica*) were obtained from a commercial supplier and were maintained at Marineland, FL, for 23 days. The oysters were then held at the laboratory for 4 days in unfiltered seawater (22°C). The oysters had a mean length of 40.1 ±5.3 mm (range of 34 to 50 mm) and mean wet weight of 0.77 g.

B. **Test System:** The test system was a continuous flow diluter system. The test vessels were 16-l glass chambers containing 8.5 l of test solution. The solution depth was 8 cm. A flow rate of 21 l/hour was provided to each test chamber.

The test was conducted at 20-21°C under a 16-hour light and 8-hour dark photoperiod.

The dilution water was unfiltered seawater from an inlet of the Atlantic Ocean. The dilution water had a salinity of 34 parts per thousand (ppt) and a pH of 7.9 at test initiation.

Various stock solutions were prepared by mixing the appropriate amount of test material with seawater.

C. **Dosage:** Ninety-six-hour flow-through acute test. The following nominal test concentrations of AC 243,997 were selected for this study: 25.9, 43, 72, 120, and 200 mg/l. A dilution water control was also included.

D. **Design:** Just prior to test initiation, oysters demonstrating new shell growth were selected for the study and 2-5 mm of new shell growth of each oyster was removed. The test was initiated when 20 oysters were indiscriminately distributed to each test chamber (one chamber/treatment). The organism loading rate was 0.031 g/l/day.

Dissolved oxygen concentration, temperature, and pH were measured daily in each chamber. Salinity in the control chamber was monitored daily.

Analytical determination of AC 243,997 using high performance liquid chromatography was performed on

filtered samples of each treatment solution collected at 0, 48, and 96 hours.

E. **Statistics:** Differences in new shell growth between the control and exposure levels were determined by analysis of variance (ANOVA) and Dunnett's multiple comparison test.

12. **REPORTED RESULTS:** Mean measured concentrations were 21.5, 42.4, 65.5, 109, and 173 mg a.i./l (Table 3-1, attached). The measured concentrations averaged 83-99% of nominal concentrations. At 48 hours in the 72 mg/l nominal concentration, "the measured concentration rose to 338 mg/L due to a partial blockage of the dilution water delivery tube. Calculations indicated that the flow rate was only reduced for a few hours. The blockage was cleared and the treatment resampled 8 hours later. The concentration of the second sample is reported" (as 64.5 mg/l).

Following 96 hours of exposure, no mortality was observed in the controls or any test concentration. Based on new shell growth and mean measured concentrations, the 96-hour  $EC_{50}$  was >173 mg a.i./l (Table 3-2 and raw data, attached). The NOEC was 109 mg a.i./l since concentrations  $\leq 109$  mg a.i./l showed no significant reduction in new shell growth when compared to the new shell growth of the control oysters.

During the test period, pH was 6.6-8.0, temperature was 20-21°C, and the salinity ranged from 34 to 35 ppt. The dissolved oxygen concentration was  $\geq 7.2$  mg/l ( $\geq 99\%$  of saturation). "There was a correlation with pH and test concentration; the higher the concentration, the lower the pH. The effect observed at 173 mg/L may have been a response to the lower pH rather than directly to the test substance."

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions, other than those mentioned above, were presented in the report.

Quality assurance and good laboratory practice statements were included in the report, indicating that the study was conducted in accordance with the EPA Good Laboratory Practice Regulations (40 CFR 160).

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures were generally in accordance with the SEP, except for the following:

Control oysters demonstrated insufficient growth (i.e., 1.36 mm new shell deposition) by test termination. As stated in an amendment to the SEP (dated 9/90), a minimum of 2 mm of shell deposition is required.

In this study, the flow rate of the test solution to each test chamber was 1.05 l/oyster/hour and no supplemental food was added. According to protocols recommended by the SEP (APHA, 1981 and EPA, 1976) each oyster should receive a minimum of 5 l of "once-through" flow through test solution per hour.

The report did not indicate whether 15- to 30-minute dawn/dusk simulation periods were provided during the study.

The continuous temperature in at least one test chamber was not monitored as recommended.

- B. **Statistical Analysis:** No concentration caused  $\geq 50\%$  reduction in new shell growth, therefore, no  $EC_{50}$  calculation was necessary.

Homogeneity of variance and normality of the data were tested using Hartley test and chi-square test, respectively. The reviewer determined the NOEC using ANOVA with Dunnett's test. The reviewer's analysis demonstrated a significant difference from the control at the highest test level (173 mg a.i./l), therefore, the NOEC was 109 mg a.i./l which is same as the NOEC presented by the author.

- C. **Discussion/Results:** This study is not scientifically sound and does not meet the guideline requirements for a 96-hour flow-through mollusc shell deposition study. The control oysters did not exhibit sufficient shell deposition as required by amendments to the guidelines (2 mm minimum). This may have been caused by the low flow rate through the system. Recommended protocols require 5 l of water/oyster/hour. The actual flow rate was 1.05 l/oyster/hour which is 21% of the recommended flow rate. Supplemental food was not provided.

Based on mean measured concentrations, the  $EC_{50}$  for eastern oysters exposed to AC 243,997 was  $>173$  mg a.i./l, therefore, AC 243,997 is classified as practically non-toxic to *Crassostrea virginica*. The NOEC was 1.09 mg a.i./l when comparing new shell growth of the treatment oysters to that of the control oysters.

D. Adequacy of the Study:

(1) **Classification:** Invalid.

*Supplemental*

*Upgraded as per  
EPA review under  
barcode D199343.*

(2) **Rationale:** Flow to the test organisms was less than recommended and no supplemental diet was provided. Control oysters demonstrated insufficient growth.

*08/23/95  
Cw/k*

(3) **Repairability:** No.

15. COMPLETION OF ONE-LINER: Yes, June 4, 1992.

Pages 6-8 \* Claimed confidential by submitter\*

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IMAZAPYR: Mean New Shell Deposition of Exposed Oysters  
File: b:\41315802.oys Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	8.040	29.040	45.840	29.040	8.040
OBSERVED	8	25	56	22	9

Calculated Chi-Square goodness of fit test statistic = 4.6354

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

IMAZAPYR: Mean New Shell Deposition of Exposed Oysters  
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Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 1.48

Closest, conservative, Table H statistic = 6.4 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 15

Actual values ==> R (# groups) = 6, df (# avg reps-1) = 19.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

IMAZAPYR: Mean New Shell Deposition of Exposed Oysters  
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	4.691	0.938	3.350
Within (Error)	114	31.922	0.280	
Total	119	36.613		

Critical F value = 2.37 (0.05,5,60)  
 Since  $F > \text{Critical } F$  REJECT  $H_0$ :All groups equal

IMAZAPYR: Mean New Shell Deposition of Exposed Oysters  
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DUNNETTS TEST - TABLE 1 OF 2  $H_0$ :Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	1.360	1.360		
2	21.5 mg/l	1.245	1.245	0.687	
3	42.4 mg/l	1.230	1.230	0.777	
4	65.5 mg/l	1.200	1.200	0.956	
5	109 mg/l	1.175	1.175	1.106	
6	173 mg/l	0.735	0.735	3.735	*

Dunnett table value = 2.28 (1 Tailed Value,  $P=0.05$ ,  $df=60,5$ )

IMAZAPYR: Mean New Shell Deposition of Exposed Oysters  
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DUNNETTS TEST - TABLE 2 OF 2  $H_0$ :Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	20			
2	21.5 mg/l	20	0.382	28.1	0.115
3	42.4 mg/l	20	0.382	28.1	0.130
4	65.5 mg/l	20	0.382	28.1	0.160
5	109 mg/l	20	0.382	28.1	0.185
6	173 mg/l	20	0.382	28.1	0.625



TITLE: IMAZAPYR: Mean New Shell Deposition of Exposed Oysters  
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 TRANSFORM: NO TRANSFORM

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	2.0000	2.0000
1	CONTROL	2	1.1000	1.1000
1	CONTROL	3	1.2000	1.2000
1	CONTROL	4	2.0000	2.0000
1	CONTROL	5	1.0000	1.0000
1	CONTROL	6	1.4000	1.4000
1	CONTROL	7	1.7000	1.7000
1	CONTROL	8	1.2000	1.2000
1	CONTROL	9	1.1000	1.1000
1	CONTROL	10	1.2000	1.2000
1	CONTROL	11	1.0000	1.0000
1	CONTROL	12	0.8000	0.8000
1	CONTROL	13	2.5000	2.5000
1	CONTROL	14	1.0000	1.0000
1	CONTROL	15	0.7000	0.7000
1	CONTROL	16	1.5000	1.5000
1	CONTROL	17	1.2000	1.2000
1	CONTROL	18	0.9000	0.9000
1	CONTROL	19	2.3000	2.3000
1	CONTROL	20	1.4000	1.4000
2	21.5 mg/l	1	0.5000	0.5000
2	21.5 mg/l	2	1.2000	1.2000
2	21.5 mg/l	3	1.4000	1.4000
2	21.5 mg/l	4	0.9000	0.9000
2	21.5 mg/l	5	1.0000	1.0000
2	21.5 mg/l	6	1.3000	1.3000
2	21.5 mg/l	7	2.1000	2.1000
2	21.5 mg/l	8	1.4000	1.4000
2	21.5 mg/l	9	2.0000	2.0000
2	21.5 mg/l	10	1.6000	1.6000
2	21.5 mg/l	11	2.0000	2.0000
2	21.5 mg/l	12	1.0000	1.0000

2	21.5 mg/l	13	1.4000	1.4000
2	21.5 mg/l	14	0.5000	0.5000
2	21.5 mg/l	15	1.0000	1.0000
2	21.5 mg/l	16	1.6000	1.6000
2	21.5 mg/l	17	2.0000	2.0000
2	21.5 mg/l	18	1.0000	1.0000
2	21.5 mg/l	19	1.0000	1.0000
2	21.5 mg/l	20	0.0000	0.0000
3	42.4 mg/l	1	0.5000	0.5000
3	42.4 mg/l	2	1.6000	1.6000
3	42.4 mg/l	3	1.4000	1.4000
3	42.4 mg/l	4	0.9000	0.9000
3	42.4 mg/l	5	1.0000	1.0000
3	42.4 mg/l	6	1.3000	1.3000
3	42.4 mg/l	7	1.8000	1.8000
3	42.4 mg/l	8	1.7000	1.7000
3	42.4 mg/l	9	1.0000	1.0000
3	42.4 mg/l	10	0.7000	0.7000
3	42.4 mg/l	11	1.1000	1.1000
3	42.4 mg/l	12	1.2000	1.2000
3	42.4 mg/l	13	1.2000	1.2000
3	42.4 mg/l	14	1.9000	1.9000
3	42.4 mg/l	15	0.0000	0.0000
3	42.4 mg/l	16	2.2000	2.2000
3	42.4 mg/l	17	1.3000	1.3000
3	42.4 mg/l	18	1.4000	1.4000
3	42.4 mg/l	19	1.0000	1.0000
3	42.4 mg/l	20	1.4000	1.4000
4	65.5 mg/l	1	0.7000	0.7000
4	65.5 mg/l	2	1.6000	1.6000
4	65.5 mg/l	3	1.2000	1.2000
4	65.5 mg/l	4	0.9000	0.9000
4	65.5 mg/l	5	1.4000	1.4000
4	65.5 mg/l	6	1.2000	1.2000
4	65.5 mg/l	7	1.0000	1.0000
4	65.5 mg/l	8	0.6000	0.6000
4	65.5 mg/l	9	1.1000	1.1000
4	65.5 mg/l	10	1.4000	1.4000
4	65.5 mg/l	11	1.2000	1.2000
4	65.5 mg/l	12	2.0000	2.0000
4	65.5 mg/l	13	1.1000	1.1000
4	65.5 mg/l	14	0.7000	0.7000
4	65.5 mg/l	15	2.0000	2.0000
4	65.5 mg/l	16	0.0000	0.0000
4	65.5 mg/l	17	1.0000	1.0000
4	65.5 mg/l	18	1.2000	1.2000
4	65.5 mg/l	19	0.9000	0.9000
4	65.5 mg/l	20	2.8000	2.8000
5	109 mg/l	1	2.0000	2.0000
5	109 mg/l	2	1.0000	1.0000
5	109 mg/l	3	0.9000	0.9000
5	109 mg/l	4	1.2000	1.2000
5	109 mg/l	5	1.4000	1.4000
5	109 mg/l	6	1.4000	1.4000
5	109 mg/l	7	1.0000	1.0000
5	109 mg/l	8	0.9000	0.9000
5	109 mg/l	9	0.0000	0.0000
5	109 mg/l	10	1.0000	1.0000
5	109 mg/l	11	0.8000	0.8000
5	109 mg/l	12	2.6000	2.6000

3	109 mg/l	13	0.6000	0.6000
5	109 mg/l	14	1.3000	1.3000
5	109 mg/l	15	1.7000	1.7000
5	109 mg/l	16	1.2000	1.2000
5	109 mg/l	17	1.1000	1.1000
5	109 mg/l	18	1.4000	1.4000
5	109 mg/l	19	0.8000	0.8000
5	109 mg/l	20	1.2000	1.2000
6	173 mg/l	1	1.0000	1.0000
6	173 mg/l	2	0.9000	0.9000
6	173 mg/l	3	0.8000	0.8000
6	173 mg/l	4	1.1000	1.1000
6	173 mg/l	5	0.6000	0.6000
6	173 mg/l	6	0.7000	0.7000
6	173 mg/l	7	0.5000	0.5000
6	173 mg/l	8	1.1000	1.1000
6	173 mg/l	9	0.0000	0.0000
6	173 mg/l	10	1.6000	1.6000
6	173 mg/l	11	0.0000	0.0000
6	173 mg/l	12	0.9000	0.9000
6	173 mg/l	13	1.0000	1.0000
6	173 mg/l	14	1.0000	1.0000
6	173 mg/l	15	0.5000	0.5000
6	173 mg/l	16	0.0000	0.0000
6	173 mg/l	17	1.6000	1.6000
6	173 mg/l	18	0.0000	0.0000
6	173 mg/l	19	0.4000	0.4000
6	173 mg/l	20	1.0000	1.0000

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Study/Species/Lab/ MRID #	Chemical X a.i.	Results	Reviewer/ Date	Validation Status
48-Hour EC <sub>50</sub>		EC <sub>50</sub> - _____ ppm ( <u>95% C.L.</u> ) Control Mortality (X) - _____ Solvent Control Mortality (X) - _____		
Species:		Slope - _____ # Animals/Level - _____ Temperature - _____		
Lab:				
MRID #		48-Hour Dose Level ppm / (X Effect) ( ) , ( ) , ( ) , ( ) , ( )		

Comments:

96-Hour EC<sub>50</sub> 99.5% LC<sub>50</sub> - 740 ppm ( NA ) 95% C.L. Control Mortality (X) - 0  
173 Solvent Control Mortality (X) - NA

Species: Cassia tora virginica Slope - NA # Animals/Level - 20 Temperature - 20.21  
Lab: Environmental Science Effect  
and Engineering 96-Hour Dose Level ppm / (X Mortality)  
21.5 ( 8 ), 42.4 ( 10 ), 65.5 ( 12 ), 109 ( 13 ), 173 ( 49 )

MRID # 413158-02 Comments: Based on mean measured conc.  
Invalid  
6/4/92